LYSIS OF SOIL FUNGI BY BACTERIA[1]

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Abstract

A number of bacteria capable of lysing *Fusarium oxysporum* were isolated from soil. One of these, a strain of *Bacillus cereus*, was studied in detail. Living and dead fusarium mycelium as well as cell-wall preparations were digested by the bacterium. Chitin and the hyphae of a number of other fungi also served as carbon sources for the active organisms, but there was no lysis of species of *Pythium*, *Streptomyces*, *Agrobacterium*, or *Pseudomonas*. Lysis appeared to be associated with chitinase and laminarinase activity of *B. cereus*, but incubation of fungus mycelium with chitinase alone, or with chitinase in combination with laminarinase, proteases and cellulase, did not result in lysis. N-Acetylhexosamine appeared during the digestion. Evidence was obtained to suggest that lytic microorganisms could destroy fungal mycelium in sterile soil.

Introduction

Predation and parasitism among microorganisms are common in both aquatic and terrestrial environments. For example, protozoa feed upon bacteria, and the decline in numbers of bacteria in a heavily manured soil was reported to be directly correlated with an increase in the abundance of protozoa (9). Anscombe and Singh (1) observed that more than half of the soil bacteria were edible to amoebae, myxobacteria, and myxomycetes. Digestion of bacteria by other bacteria or by actinomycetes is also well documented. Richmond (21) isolated a strain of *Bacillus subtilis* which produced extracellular enzymes capable of lysing *Micrococcus lysodeikticus*, and Salton (22) noted that certain species of *Streptomyces* digested cell-wall preparations of various bacteria. A species of *Streptomyces* was likewise shown to degrade yeast cell walls (10).

Lysis of fungi by bacteria has also been occasionally noted. Thus, an isolate of *Bacillus macerans* was observed to digest *Fusarium roseum* (19), and lysis of *Aspergillus oryzae* by a strain of *Bacillus circulans* was demonstrated by Horikoshi and Iida (12). Little is known of the mechanism of the microbiological digestion of fungus hyphae or of the ecological significance of this process. The present study is concerned with establishing characteristics of the mycolytic flora of soil, i.e., those microorganisms capable of effecting the digestion of fungus mycelium.

Materials and Methods

Mycolytic bacteria were isolated by a modification of the method of Mueller and Durrell (18). Viable *Fusarium oxysporum* f. *cubense* mycelium in water agar (1.5% agar in tap water) was placed in perforated cellulose acetate test tubes having a diameter of 18 mm. The tubes were inserted into samples of 12 Central American soils and in Honeoye silt loam for a period of 2 weeks.

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A description of these soils has been given elsewhere (15). Mycolytic bacteria were isolated from the agar and transferred to an inorganic salts medium with either living or dead fungus hyphae as the sole carbon source. The basal medium contained KNO_3, 4.0 g; KH_2PO_4, 0.03 g; $MgSO_4.7H_2O$, 0.6 g; $FeCl_3$-$.6H_2O$, 0.05 g; $ZnSO_4.7H_2O$, 0.03 g; and distilled water, 1.0 liter. The carbon sources were added to give a final concentration of 0.1% calculated on a dry weight basis. When the basal medium was used for the cultivation of *Bacillus cereus*, yeast extract (Difco) was added to a concentration of 0.001%. Growth of the bacterium in the yeast extract enriched medium was insignificant in the absence of the added carbon source.

The mycelium was killed either by steaming at 100° C for 20 minutes or by immersion of the fungus suspension in 95% ethanol for a period of 2 hours. The mycolytic isolates were able to degrade the mycelium treated by the two different procedures. Cell-wall preparations of *F. oxysporum* were made by the alkaline digestion method of Aronson and Machlis (2).

The medium used for the culture of *F. oxysporum* was identical with that used for the mycolytic isolates except for the inclusion of 40 g sucrose per liter. When the mycelium collected from this medium was to be used as a substrate, it was ground in a mortar and washed twice with distilled water by centrifugation. Lysis was detected by measurement of the change in optical density of the suspension at a wavelength of 625 mμ. Incubation was at room temperature. The formation of N-acetylhexosamine was determined colorimetrically by the procedure of Reissig, Stominger, and Leloir (20).

For the determination of laminarin, the fungus cell walls were extracted at room temperature for 24 hours with 5.0% HCl and the resultant extract precipitated with 95% ethanol. This extraction process was repeated 3 times. To detect the presence of laminarin, the iodimetric method of Connell, Hirst, and Percival (8) was applied to the partially purified fraction. The results of the chemical test were reinforced by examination of the ability of laminarinase-producing microorganisms to use the preparation as sole carbon source. The utilization by bacterial isolates of a purified laminarin prepared from laminaria as the sole carbon source in inorganic media was considered to be evidence for the formation of laminarinase.

The decline of *F. oxysporum* in artificially infested soil was investigated by the plate-count technique. A suspension of the fusarium consisting largely of conidia was added to Mopala loam, and the number of viable units of the fungus remaining in the soil was determined by enumeration on Martin's medium (17). Parallel counts of *F. oxysporum* were made in uninfested Mopala loam at each time interval and the fungal abundance expressed as the difference between the two estimates. The moistened soil was incubated in tumblers at 25° C throughout the investigation. Results are the means of three replicate soil samples in three separate tumblers. Bacillus, pseudomonas, and actinomycetes were enumerated on gelatin according to the method of Conn (7).

The culture of *Pseudomonas chitinovorans* was kindly provided by Dr. H. Veldkamp, Laboratorium voor Microbiologie, Landbouwhogeschool, Wageningen, Netherlands. Trypsin, pepsin, and cellulase were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Chitin was provided

by Moretex Chemical Corporation, Spartanburg, South Carolina, and laminarin was obtained from the Institute of Seaweed Research, Musselburgh, Midlothian, Scotland. Dried laminaria was obtained from the Marine Biological Laboratory, Woods Hole, Massachusetts.

Results

Enrichments for organisms lytic to *F. oxysporum* were prepared with aliquots from each of the 12 Central American soils and the Honeoye silt loam. From these enrichments, five bacteria capable of digesting the fungus mycelium were obtained in pure culture. The soils from which each of these bacteria were isolated as well as the genus and species description of each bacterium are shown in Table I. The bacteria active on the test fungus were limited to strains of *Bacillus* and *Pseudomonas*, but the technique of enrichment might have favored specific microbial groups over others which may be active in the natural environment.

TABLE I

Relation between mycolysis and the formation of chitinase and laminarinase

Microorganism	Source	Lysis of *Fusarium oxysporum*	Formation of	
			Chitinase	Laminarinase
Bacillus cereus OS2	Flooded San Alejo loam	+	+	+
Bacillus cereus A	Mopala loam	+	+	+
Bacillus megaterium 8A	Caoba loam	+	+	+
Bacillus megaterium 9A	Caoba silty loam	+	+	+
Pseudomonas sp. PA	Honeoye silt loam	+	+	+
Pseudomonas sp. PB	Williamson silt loam	−	+	+
Rhizopus sp. RA	Williamson silt loam	−	+	+

The lytic bacteria isolated by the enrichment procedure were examined to determine whether they would grow in an inorganic salts medium containing either living or dead fusarium as the sole carbon source. Only liquid media were employed since it had been noted earlier that the use of agar media could give erroneous results because of the microbiological utilization of some component of the commercial agar as carbon source (16). When tested in these solution cultures, each of the bacteria developed at the expense of the hyphae supplied as substrate.

One of the lytic organisms, *B. cereus* strain OS2 isolated from flooded San Alejo loam, was selected for further study. It was found to be capable of utilizing cell-wall preparations of *F. oxysporum* as a sole carbon source and also living or dead hyphae. For total lysis of a washed preparation of live *F. oxysporum* mycelium (added to the inorganic salts medium to give a concentration of 0.1%), a period of 7 to 10 days was required. Free N-acetylhexosamine did not accumulate during the initial stages of digestion, but it was detected after about 7 days.

Filtrates of *B. cereus* cultures, grown in the basal medium with killed fusarium as the carbon source, were freed of bacterial cells by passage through

an ultrafine sintered glass filter. These filtrates were capable of causing lysis of living and dead hyphae (Table II). The filtrate was more active against

TABLE II

Lysis of living and killed *Fusarium oxysporum* by culture filtrates of a lytic strain of *Bacillus cereus*

	% initial optical density at 625 mμ	
Time, hours	Live fusarium	Dead fusarium
0.0	100	100
0.5	89.0	94.7
1.0	76.0	87.4
2.0	69.5	80.3

NOTE: No lysis of the fungus was noted in the absence of the lytic preparation.

living than killed *F. oxysporum*. No free N-acetylhexosamine accumulation was observed during the digestion. Cold ammonium sulphate (final concentration 70% of saturation) added to the lytic filtrates yielded a precipitate which, when dissolved in 0.01 *M* phosphate buffer at pH 7.0, catalyzed an active lysis of dead hyphae. A decline in optical density of 20% was observed in 5 hours with a preparation made up of 1 part of precipitate to 1000 parts of buffer. The lytic activity was destroyed when culture filtrates were heated for 15 minutes at 60° C.

The mycolytic activity of *B. cereus* decreased slightly after five sub-cultures in nutrient broth (Difco). When this partially attenuated organism was grown for 24 hours on young hyphae of *F. oxysporum*, incomplete lysis was noted, and spheroplast-like bodies were released. These spherical bodies lost their structural integrity within 2 hours after their release from the lysing filaments. An initial stage in the formation of these spheroplast-like structures is shown in Fig. 1. The diameter of the spherical body was much greater than the width of the hypha, either at the time of the initial liberation or at the subsequent period when the spheroplast existed free in the liquid. A similar increase in size of spheroplasts immediately after release from the fungal cell was noted by Bachman and Bonner (3) with *Neurospora crassa*.

Fusaria are reported to contain chitin as a constituent of their cell walls (24); consequently, an attempt was made to associate the digestion with the possible formation and activity of chitinase by the lytic bacteria. All five lytic microorganisms grew well in an inorganic salts medium containing chitin as a sole carbon source, and free N-acetylhexosamine appeared in the supernatant during such development. However, there was no lysis of *F. oxysporum* by culture filtrates of chitin-grown *B. cereus*. Furthermore, a culture filtrate obtained from *P. chitinovorans* grown on chitin for 14 days at 30° C did not lyse *F. oxysporum* nor did it release N-acetylhexosamine when incubated with the fungus; on the other hand, the filtrate contained chitinase as measured by N-acetylhexosamine production from purified chitin. Negative results were also obtained when mycelium was incubated with a crude chitinase prepared from an extract of table mushroom by the method of Carlisle (5). It is thus unlikely that chitinase alone is responsible for lysis.

PLATE I

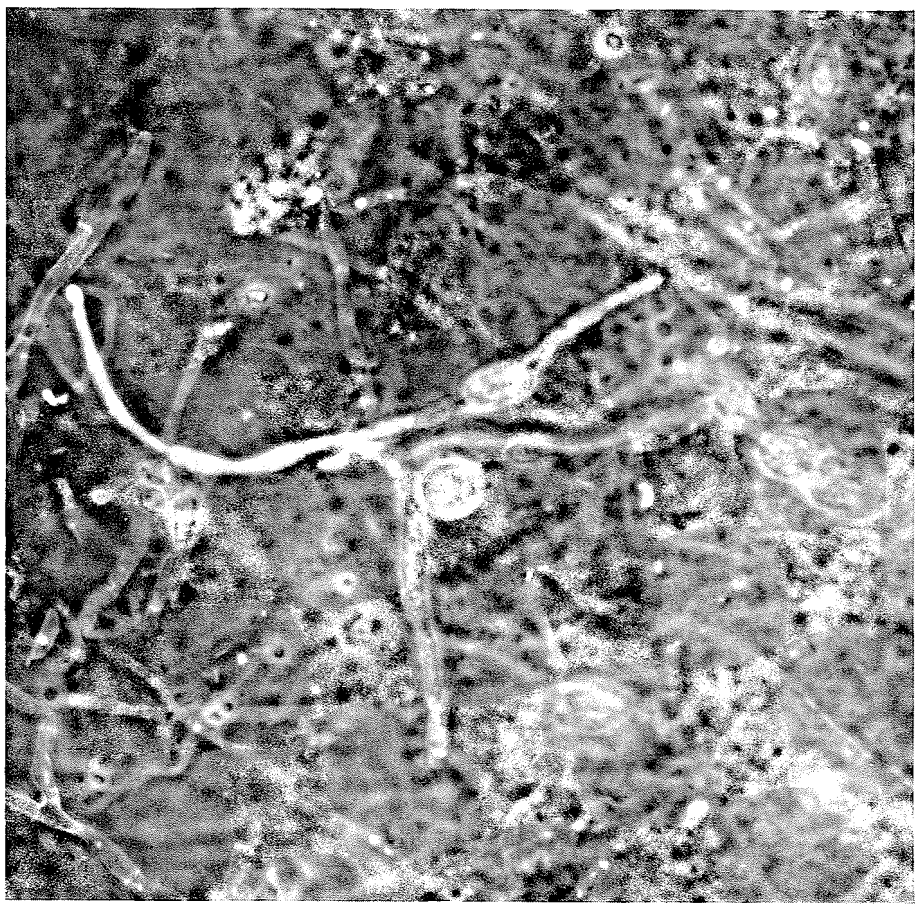

FIG. 1. Release of spheroplast-like bodies from hyphae of *Fusarium oxysporum*.

As fungal cell walls may contain protein and cellulose, a study was made to determine whether proteolytic and cellulolytic enzymes might participate in the digestion of the walls. Mycelium harvested from a 2-day shake culture of *F. oxysporum* was killed by immersing the hyphae in 95% ethanol. The mycelium was then washed and suspended in 10 ml of a 0.01% solution of trypsin made up in 0.01 *M* phosphate buffer, pH 6.0, or in a pepsin solution made up in 0.01 *M* citrate buffer, pH 2.0. After a 4-hour incubation at 30° C, 10 ml of a solution containing cellulase and mushroom chitinase, 0.01% of each in 0.01 *M* phosphate buffer, pH 7.0, were added. Before the addition of the two carbohydrases, the proteolytic enzyme was inactivated by heating the mixture at 100° C for 15 minutes. The influence of reversing the order of enzyme treatment was also investigated, i.e., the mixture of chitinase and cellulase was incubated with the fungus for 4 hours prior to addition of the proteolytic enzymes. However, lysis was not observed as a result of exposure of the hyphae to any of the various combinations.

Tests were carried out to determine whether laminarin, a β-1,3-glucan, was present in cell walls of the fungi. The glucan was detected in mycelium of both *F. oxysporum* f. *cubense* and *Fusarium solani* f. *phaseoli*. All five isolates of lytic bacteria formed a β-1,3-glucanase capable of decomposing laminarin (Table I). Bacterial laminarinase, either alone or in combination with chitinase and proteases, did not decompose fungal hyphae. Furthermore, a *Rhizopus* sp. and a *Pseudomonas* sp. which had been isolated by inoculating soil into a minimal medium containing either 0.1% chitin or 0.1% laminarin followed by plating, formed both chitinase and a β-1,3 glucanase, but neither of these microorganisms displayed any lytic activity toward *F. oxysporum* (Table I). It would appear from the data that either these enzymes bear no relation to the lytic process or that some additional factor or enzyme has been omitted from the incubation mixture.

In view of the possible chemical similarities of the cell walls of diverse fungi, the susceptibilities of other genera of filamentous fungi to bacterial attack were examined. A medium similar to that employed for observing lysis of *F. oxysporum* hyphae was used to culture the selected fungi. For comparative purposes, two bacteria, a yeast, and an actinomycete were likewise examined. The organism serving as substrate was then added to the inorganic salts solution to give a final concentration of 0.1% by weight of the living cells. Growth of the lytic bacterium and digestion of the test organism were measured both microscopically and by observing changes in optical density of the medium. Following growth of the lytic bacterium on the several fungi, culture filtrates were also prepared and tested to determine which of them would lyse the organism used as carbon source for the active *B. cereus* strain. Lysis by culture filtrates was determined by optical density measurements during an 8-hour period at room temperature.

The results summarized in Table III reveal that a range of microorganisms was attacked by *B. cereus*. Mycelia of all the test fungi were digested with one exception. A species of *Pythium*, the only fungal genus of the group investigated which does not contain strains reportedly possessing chitin in their cell walls, was apparently resistant to attack. Laminarin was also apparently absent from cell-wall preparations of *Pythium debaryanum*. There did appear

TABLE III

Microorganisms digested by a lytic strain of *Bacillus cereus*

Organism	Lysis	Chitin in cell walls*
Fusarium oxysporum f. *cubense*	+	+
Fusarium oxysporum f. *conglutinans*	+	+
Fusarium solani f. *phaseoli*	+	+
Penicillium sp.	+	+
Aspergillus sp.	+	+
Rhizoctonia sp.	+	?
Thielaviopsis sp.	+	?
Pythium debaryanum	−	−
Stemphylium sp.	+	?
Alternaria sp.	+	+
Mucor sp.	+	+
Rhizopus nigricans	+	+
Neurospora crassa	+	+
Zygorhynchus sp.	+	?
Saccharomyces cerevisiae	−	−
Streptomyces sp.	−	−
Agrobacterium sp.	−	−
Pseudomonas sp.	−	−

*Data from Blumenthal and Roseman (4), Cochrane (6), and Thomas (24).
+ Indicates presence of chitin in one or more strains of the genus.
− Indicates that the strains examined did not contain chitin.

to be a slight degree of decomposition of the hyphae, but the change in optical density was so small that it may have been within the range of experimental error. The mycolytic *B. cereus* isolate failed to grow upon the bacteria, actinomycete, and yeast, all reportedly devoid of chitin; moreover, there was no digestion of these microorganisms by mycolytic culture filtrates of the bacterium.

The population of a pathogenic strain of *F. oxysporum* has been observed to decline rapidly following the addition of a large inoculum of the fungus to non-sterile soil (23). To determine if there was any relation between the activities of lytic bacteria and this decline, *F. oxysporum* was added to a sample of Mopala loam which had been kept moist for a period of 1 month. Counts of the fungus as well as of bacilli, pseudomonas, and actinomycetes were made at regular intervals. Emphasis was placed upon these groups in the light of previous results on the identity of the lytic bacteria and of Lockwood's (14) studies of streptomycetes. The data in Table IV indicate that

TABLE IV

Microbiological changes occurring during the decline of *Fusarium oxysporum* in infested Mopala loam

	Population × 10^3/g soil		
Time, days	Bacillus and pseudomonas	Actinomycetes	*F. oxysporum*
0	220	90	1,500
1	210	42	4,100
3	1,580	250	130
7	18,000	300	—
14	2,000	300	17

the fusarium maintained its initial population for only about 1 day, the viable count of the pathogen falling to less than 10% of the original value by the third day. By contrast, the population of bacilli and pseudomonas increased more than sevenfold during the first 3 days and reached a level almost a hundredfold higher by the seventh day. The increase in population density of the actinomycete flora, composed almost entirely of *Streptomyces* spp., was not as marked. There seems to be a relationship between the increase in abundance of these bacterial and actinomycete groups in soil and the decline of *F. oxysporum*. It is noteworthy that species of *Bacillus*, *Pseudomonas*, and *Streptomyces* are particularly active in synthesizing chitinase (25).

The observations regarding the stimulation of *Bacillus*, *Pseudomonas*, and, to a lesser extent, *Streptomyces*, during the decline of a pathogenic strain of fusarium prompted a more detailed study to determine whether lysis can take place in soil. Twenty-gram quantities of Mopala loam were sterilized in petri dishes, and the soil was inoculated with 0.1 ml of a suspension of *F. oxysporum*. In one set of plates, *B. cereus* strain OS2 was added simultaneously with the fungus. In a second series, the fungal mycelium was allowed to develop for 3 days prior to inoculation with the bacterium. The data in Table V

TABLE V

Inhibition and lysis of *Fusarium oxysporum* in sterile soil following inoculation of *Bacillus cereus*

Inoculum	Radial spread of fungus mycelium, cm		
	0 days	7 days	10 days
	Simultaneous inoculation*		
F. oxysporum alone	0	4.9	5.0
F. oxysporum + *B. cereus*	0	0	0
	Inoculation 3 days apart†		
F. oxysporum alone	1.3	5.0	5.0
F. oxysporum + *B. cereus*	2.2	0.5	0

*Both organisms added at the same time.
†*B. cereus* added 3 days after inoculation with the fungus. Zero time is here taken as the time of inoculation with the bacterium.

demonstrate that simultaneous inoculation with the bacterium resulted in a complete inhibition of the fungus. Moreover, the fungal mycelium which developed in the 3 days before the bacterium was added (second series) was rapidly digested. No mycelium was observed by visual examination of the soil of either treatment 10 days after bacterial inoculation.

Upon inoculation of the soil into Martin's (17) medium after the 10-day incubation period, 10 or fewer fungus colonies per gram of soil appeared. Hence, although the mycelium was digested in soil, the fungus did survive, possibly in the form of conidia or as the resistant chlamydospores.

Discussion

The existence in soil of microorganisms which are capable of digesting fungi suggests that mycolytic microorganisms may be active in the normal biological control of phytopathogenic fungi. The isolation of only *Bacillus* and *Pseudomonas* species does not imply that these are the only genera which

can digest soil fungi since the technique employed for isolation tended to favor rapidly growing microorganisms. Lockwood (14), for example, has presented evidence that *Streptomyces* species may be active in the destruction of soil-borne plant pathogenic fungi. The relatively rapid increase in abundance of bacilli, pseudomonas, and streptomycetes at the time of disappearance of *F. oxysporum* from non-sterile soil, moreover, lends weight to the possible importance of these genera in mycolysis in nature. It is not yet clear, however, which microbial groups are the most important.

The possession of chitinase by all of the lytic bacteria is noteworthy because the ability to form this enzyme is not a characteristic common to most soil bacteria (25). The selection for mycolytic microorganisms apparently results in a simultaneous selection of chitinoclastic strains. This correlation may be entirely fortuitous because chitinase alone or in combinations with β-1,3-glucanase, proteases, and cellulase did not bring about a destruction of the fungus cell wall. Nevertheless, the enhancement of lysis of *Aspergillus flavus* by *B. circulans* through the addition of a chitinase preparation (11) and the existence of chitin in the walls of many filamentous fungi argue strongly for a functional role for the enzyme. Furthermore, the observation that fungi containing chitin in their cell walls were lysed whereas those organisms from which chitin was absent were not, provides further evidence to support the hypothesis that there is a functional relation between mycolysis by *B. cereus* and the degradation of chitin in the hyphal wall.

The report of Horikoshi, Koffler, and Garner (13) that laminarin occurs in *Penicillium chrysogenum* cell walls prompted a similar study with the fusaria. The presence of the glucan in mycelium of both *F. oxysporum* f. *cubense* and *F. solani* f. *phaseoli* suggests that in order for a lytic microorganism to digest mycelium of these fungi, it should form the laminarinase. Despite the detection of that enzyme in lytic microorganisms, it is clear from the evidence that some other factors in addition to chitinase and the glucanase are required for digestion.

References

1. ANSCOMBE, E. J. and SINGH, B. N. Limitation of bacteria by microorganisms in soil. Nature, **161**, 140–141 (1948).
2. ARONSON, J. M. and MACHLIS, L. The chemical composition of the hyphal walls of the fungus *Allomyces*. Am. J. Botany, **46**, 292–300 (1959).
3. BACHMAN, B. J. and BONNER, D. M. Protoplasts from *Neurospora crassa*. J. Bacteriol. **78**, 550–556 (1959).
4. BLUMENTHAL, H. J. and ROSEMAN, S. Quantitative estimation of chitin in fungi. J. Bacteriol. **74**, 222–224 (1957).
5. CARLISLE, D. B. Softening chitin for histology. Nature, **187**, 1132–1133 (1960).
6. COCHRANE, V. W. Physiology of fungi. John Wiley and Sons, New York. 1958.
7. CONN, H. J. The most abundant groups of bacteria in soil. Bacteriol. Rev. **12**, 257–273 (1948).
8. CONNELL, J. J., HIRST, E. L., and PERCIVAL, E. G. V. The constitution of laminarin. Part I. An investigation of laminarin isolated from *Laminaria cloustoni*. J. Chem. Soc. 3494–3500 (1950).
9. CUTLER, D. W. and CRUMP, L. M. Problems in soil microbiology. Longmans, Green & Co., London. 1935.
10. FURUYA, A. and IKEDA, Y. Studies on the cell wall lytic enzymes produced by *Streptomyces* species. J. Gen. Appl. Microbiol. (Tokyo), **6**, 40–47 (1960).
11. HORIKOSHI, K. The effect of lytic enzymes from *Bacillus circulans* and chitinase from *Streptomyces* sp. on *Aspergillus oryzae*. Nature, **183**, 186–187 (1959).
12. HORIKOSHI, K. and IIDA, S. Lysis of fungal mycelia by bacterial enzymes. Nature, **181**, 917–918 (1958).

13. HORIKOSHI, K., KOFFLER, H., and GARNER, H. R. Demonstration of laminarin in fungal cell walls. Bacteriol. Proc. 95 (1961).
14. LOCKWOOD, J. L. *Streptomyces* spp. as a cause of natural fungitoxicity in soils. Phytopathology, **49**, 327–331 (1959).
15. MARSHALL, K. C. and ALEXANDER, M. Competition between soil bacteria and *Fusarium*. Plant Soil, **12**, 143–153 (1960).
16. MARSHALL, K. C., WHITESIDE, J. S., and ALEXANDER, M. Problems in the use of agar for the enumeration of soil microorganisms. Soil Sci. Soc. Am. Proc. **24**, 61–62 (1960).
17. MARTIN, J. P. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. Soil Sci. **69**, 215–232 (1950).
18. MUELLER, K. E. and DURRELL, L. W. Sampling tubes for soil fungi. Phytopathology, **47**, 243 (1957).
19. PARK, D. Effect of substrate on a microbial antagonism, with reference to soil conditions. Trans. Brit. Mycol. Soc. **39**, 239–259 (1956).
20. REISSIG, J. L., STOMINGER, J. L., and LELOIR, L. F. A modified colorimetric method for the determination of N-acetyl amino sugars. J. Biol. Chem. **217**, 959–966 (1955).
21. RICHMOND, H. Formation of a lytic enzyme by a strain of *Bacillus subtilis*. Biochim. Biophys. Acta, **33**, 78–92 (1959).
22. SALTON, M. R. J. Isolation of *Streptomyces* spp. capable of decomposing preparations of cell walls from various microorganisms, and a comparison of their lytic activities with those of certain other actinomycetes. J. Gen. Microbiol. **12**, 25–30 (1955).
23. STOVER, R. H. Flood-fallowing for eradication of *Fusarium oxysporum* f. *cubense*. II. Some factors involved in fungus survival. Soil Sci. **77**, 401–414 (1954).
24. THOMAS, R. C. Composition of fungal hyphae. I. The fusaria. Am. J. Botany, **15**, 537–547 (1928).
25. VELDKAMP, H. Aerobic decomposition of chitin by microorganisms. Mededel. Landbouw. Wageningen. **55**, 127–174 (1955).